Association between toenail selenium and risk of acute myocardial infarction in European men. The EURAMIC Study. European Antioxidant Myocardial Infarction and Breast Cancer.

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Abstract: The association between selenium status and risk of acute myocardial infarction was examined in a multicenter case-control study in 10 centers from Europe and Israel in 1991-1992. Selenium in toenails was assessed for 683 nonfatal male cases with first acute myocardial infarction and 729 controls less than 70 years of age. Median toenail selenium content was 0.553 g/g for cases and 0.590 g/g for controls. After adjustment for age, center, and smoking, the odds ratio for myocardial infarction in the highest quintile of selenium as compared with the lowest was 0.63 . The observed inverse trend was somewhat stronger when the authors adjusted for vitamin E status (p = 0.05). Analysis stratified for smoking habits showed an inverse association in former smokers (odds ratio for the 75th-25th percentile contrast = 0.63 (95 percent confidence interval 0.43-0.94)), but not in current smokers (odds ratio = 0.97 (0.71-1.32)) or in those who had never smoked (odds ratio = 1.55 (0.87-2.76)). Analysis stratified by center showed a significant inverse association between selenium levels and risk of myocardial infarction for Germany (Berlin) only (75th to 25th percentile odds ratio = 0.62 (95 percent confidence interval 0.42-0.91)), which was the center with the lowest selenium levels. It appears that the increased risk of acute myocardial infarction at low levels of selenium intake is largely explained by cigarette smoking; selenium status does not appear to be an important determinant of risk of myocardial infarction at the levels observed in a large part of Europe. Am J Epidemiol 1997; 145: 373-9

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Association Between Toenail Selenium and Risk of Acute Myocardial Infarction in European Men

The EURAMIC Study

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antioxidants; case-control studies; myocardial infarction; selenium

Evidence is accumulating that oxidation of low density lipoprotein particles and cytotoxic effects of lipid peroxides enhance the formation of foam cells and atherosclerotic lesions (1, 2). Selenium is part of the enzyme glutathione peroxidase, which plays an important role in the antioxidant defense of the body against the deleterious actions of free radicals and lipid peroxides. The question of whether low selenium status predisposes to cardiovascular disease has been addressed in a number of studies in the past decade (3–10). In several prospective (3–5) and case-control (6) studies, low serum selenium levels were associated with increased risk of coronary heart disease. However, in other studies, this association could not be confirmed (7–10). Antioxidant defense is achieved by additive or synergistic action of enzymes and antiox-

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Abbreviations: EURAMIC, European Antioxidant Myocardial Infarction and Breast Cancer.

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idant nutrients (11, 12). Selenium and vitamin E in particular appear to have overlapping and partly compensative functions (13–17). Therefore, it is conceivable that the relation between selenium status and risk of coronary heart disease is influenced by the status of other antioxidants, as has been observed in an animal experiment (18). This possible interactive effect has scarcely been addressed in population studies. In a prospective study, Kok et al. (8) reported no interaction between serum selenium and serum vitamin E and risk of cardiovascular death. However, in another study (19), patients with severe atherosclerosis had significantly lower plasma selenium levels (relative to polyunsaturated fatty acids) than patients with mild atherosclerosis, but only in the subgroup with low plasma vitamin E status.

We have studied the association between selenium and acute myocardial infarction in a case-control study in nine different countries. The large variation in selenium intake in these centers permits evaluation of a dose-response relation; comparison of this relation between populations will show whether it is consistent. Selenium levels were measured in toenail clippings, a biomarker of long-term intake not affected by the acute event of the disease (20–22).

MATERIALS AND METHODS

Design and participants

The European Antioxidant Myocardial Infarction and Breast Cancer (EURAMIC) Study was a multicenter international case-control study (23, 24). To evaluate the association of antioxidants with the risk of developing a myocardial infarction, a total of 742 cases and 757 controls were recruited concurrently during 1991 and 1992 in eight European countries and Israel, according to the same eligibility criteria. Eligible individuals were men less than 70 years of age, without previously reported myocardial infarction. Persons were excluded if they had a history of drug or alcohol abuse or major psychiatric disorders, if they were institutionalized, or if they had modified their dietary pattern (including supplement use) in the previous year. Cases were individuals who had received a first acute myocardial infarction diagnosis (*International Classification of Diseases*, Ninth Revision, code 410), which was confirmed by specific electrocardiographic abnormalities and elevated enzyme levels (25), and who had been admitted within 24 hours of manifesting symptoms. They were recruited from the coronary care unit of participating hospitals. Individuals without a history of myocardial infarction were eligible as controls and were recruited from the population in the catchment area and frequency matched for age according to 5-year intervals. If possible, random samples from local population registries were used (Finland, Israel, Germany, Scotland, Switzerland). In some centers (Russia, Spain), population registries could not be used due to incomplete coverage or legal restrictions. Therefore, hospital controls were selected, with diseases that were not known to be associated with antioxidant status: noninfectious prostatism, renal colic, hernia, acute appendicitis/acute mesenteric adenitis, and volvulus/subocclusion due to fibrosis, rectal/anal pathology (apart from cancer, hemorrhoids, and chronic infections).

When it was anticipated that low response rates from population-based samples would compromise internal validity, controls were selected from the catchment area via a random sample by the patient's general practitioner (Netherlands) or by inviting friends and relatives of the case (Norway). In the Netherlands, Russia, and Spain, recruitment methods were combined. The overall response rates were 81 percent in cases and 64 percent in controls (table 1). Informed consent was obtained in accordance with the ethical standards of the responsible committees on medical research.

Information on smoking habits, history of hypertension, angina pectoris, and diabetes was collected by standard questionnaires (26). Socioeconomic status, family history, and alcohol intake were assessed through locally developed questionnaires. Smoking status was recorded as current, ex-, or never smoker; for current smokers, the daily number of cigarettes smoked was asked. Family history of coronary heart disease was defined as mother, father, or sibling having died of myocardial infarction or other heart disease, without specification of the person's age when the event occurred.

A nonfasting blood sample was drawn not later than 24 hours after onset of symptoms. Serum total and

TABLE 1. Population response rate and method of control recruitment in the EURAMIC Study, 1991–1992

Center	Cases (response %)	Controls (response %)	Method of control recruitment*
Finland (Helsinki)	62 (97)	61 (51)	PR
Germany (Berlin)	77 (82)	97 (73)	PR
Israel (Jerusalem)	59 (60)	60 (53)	PR
Netherlands (Zeist)	72 (75)	63 (50)	GP, PR
Norway (Sarpsborg)	101 (96)	102 (98)	FR
Russia (Moscow)	100 (97)	100 (79)	GP, H
Scotland (Edinburgh)	58 (98)	43 (61)	PR
Spain (Granada)	57 (45)	54 (67)	H
Spain (Malaga)	100 (89)	102 (77)	H, GP
Switzerland (Zürich)	57 (93)	74 (26)	PR

* PR, population register; GP, general practitioners; H, hospital controls; FR, friends, relatives.

high density lipoprotein cholesterol levels were determined enzymatically with a Boehringer Mannheim kit (Boehringer Mannheim GmbH, Mannheim, Germany) at the National Public Health Institute in Helsinki, Finland.

Subcutaneous adipose tissue was taken from the buttock by needle aspiration (27). In cases, the adipose sample was taken within 7 days of hospital admission. Samples were stored at −70°C and analyzed in a central laboratory. Alpha-tocopherol and beta-carotene were determined in the adipose tissue after saponification by reverse-phase high performance liquid chromatography and spectrophotometric detection (28), with an overall coefficient of variation of 7 percent. Vitamin concentrations in adipose tissue were expressed in μg/g of total fatty acids. Fatty acid content was assessed by gas-liquid chromatography (29) in an aliquot of the same extract as that of the vitamins, adding heptadecanoic acid (C17:0) as an internal standard to the sample before saponification.

Selenium in toenails

Toenail clippings from all 10 toes were collected within 8 weeks of inclusion in the study and were stored in small plastic bags at room temperature. Large nail clippings were cut into pieces smaller than 9 mm. The selenium content of the toenails (mean weight (standard deviation) = 54 (39 mg)) was assessed by a single measurement by instrumental neutron activation analysis of the metastable-selenium-77 isotope at a central laboratory (Interfaculty Reactor Institute, Technical University, Delft, The Netherlands) (30) by personnel blinded to disease status, over a period of 2 years. Per center, samples from cases and controls were analyzed together and randomly distributed over batches. Samples were irradiated for 17 seconds in a thermal flux of 1.1×10^{17} neutrons/second/cm^2. After a decay time of 20 seconds, gamma radiation of ^{77m}Se was measured for 30 seconds. Samples were rotated during irradiation.

The mean level of selenium (n = 87) in certified bovine liver reference material (NBS-1577A) was 0.76 ± 0.04 ppm (coefficient of variation = 5.3 percent) against a certified value of 0.71 ± 0.07 ppm. Per batch of 50 samples, two standard reference samples were included.

Selenium concentrations were reported in ppm (μg/g), and for each sample, an error percentage was reported as an estimate of the accuracy of the given value. The error percentage depends on errors in the measurement of peak areas, uncertainty in the neutron flux, and uncertainty in the nuclear constants used. For samples less than 20 mg (n = 196, mean 13 mg ± 4), the error percentage was 12.3 percent ± 4.5; for samples of 20 mg or more, the error percentage was 7.4 percent ± 4.0. The limit of detection was defined as the concentration at which selenium could be detected with 97.5 percent certainty. The limit of detection depends on the sample weight. For a sample of 20 mg, the detection limit was 0.400 ppm; for a sample of average weight (54 mg), the detection limit was 0.170 ppm.

Data analysis

Toenail clippings were not available for 55 cases and 23 control individuals. In another nine samples (four cases, five controls), selenium values were found to be below the detection level; these were excluded from data analysis. The prevalence of risk factors of coronary heart disease among those excluded was similar to that among the included subjects. Included were the results of 1,412 persons (683 cases and 729 controls). From 1,309 of these individuals, sufficient adipose tissue samples could be obtained for reliable assessment of alpha-tocopherol and beta-carotene. For 11 of the 1,412 persons, information about smoking habits was incomplete; they were excluded from multivariate analyses.

Crude means for major risk factors and potential confounders among cases and controls were calculated and the difference in means were tested with Student's t test and chi-square analyses. Median levels of selenium (and the 25th and 75th percentiles) among cases and controls were computed from the center-specific distributions. The associations between potential risk factors and myocardial infarction in the overall control group were estimated with Pearson and Spearman correlations and analysis of variance. Selenium values were log transformed to normalize a skewed distribution; retransformed values are presented where applicable. Smoking categories included never smokers, exsmokers, and current smokers. Exsmokers were defined as having stopped smoking 2 or more years before entering the study. In the logistic regression models, a variable representing these smoking categories as well as a continuous variable for the number of cigarettes smoked daily (zero for never and former smokers) were included. For multivariate analysis, multiple logistic regression was used with maximum likelihood estimation of the regression coefficients and their standard errors. Per center, the age- and smoking-adjusted odds ratio of the contrast in selenium level between the 75th and 25th percentile (among controls) was calculated, with selenium and age as continuous variables in the logistic regression model. The overall data were also stratified for smoking (current, ex-, and never smokers), and the continuous odds ratio was estimated, adjusting for age, center, and, for current

smokers, the number of cigarettes smoked. For the pooled data, odds ratios were also calculated for the highest quintile compared with the lowest, based on the distribution among controls from all centers combined. Tests for trend were performed by assigning each subject the median value for the category and treating this value as a continuous variable in the model (31). All analyses were performed with the BMDP statistical software package.

RESULTS

The prevalence of coronary heart disease risk factors in myocardial infarction cases and controls is given in table 2. Significant crude differences are observed for age, history of hypertension, smoking, angina pectoris, diabetes mellitus, family history of coronary heart disease, and body mass index. Lower total serum cholesterol concentrations in cases are almost certainly due to the acute effect of the myocardial infarction. Median selenium concentrations in toenails for cases and controls in the different centers are shown in table 3. The overall median is 0.553 μg/g in cases and 0.590 μg/g in controls. Highest median level (in Israel) and lowest median level (in Germany) varied by a factor of almost 2.

The relation of selenium concentration to coronary heart disease risk factors was examined in controls. Persons who currently smoked cigarettes had significantly lower selenium levels (0.553 μg/g) than ex-smokers (0.599 μg/g) and those who had never smoked (0.652 μg/g). The Spearman correlation between the number of cigarettes smoked daily (among current smokers only) and selenium was −0.22 ($p < 0.01$). Individuals with a family history of coronary heart disease had significantly higher selenium levels than those without a family history of coronary heart disease (0.623 μg/g and 0.579 μg/g, respectively). Selenium concentration was not associated with age, total cholesterol, body mass index, angina pectoris, diabetes, alcohol use, or hypertension. There was either no correlation or a weak correlation between vitamin E or beta-carotene levels and toenail selenium ($r = -0.04$, $p = 0.25$; $r = 0.08$, $p = 0.03$, respectively).

TABLE 2. Risk factors for coronary heart disease in cases with acute myocardial infarction and in control subjects in the EURAMIC Study, 1991–1992

Risk factor	Cases ($n = 683$)		Controls ($n = 729$)	
	Mean	(SD†)	Mean	(SD)
Age (years)	54.7	(8.9)	53.2*	(9.2)
Serum cholesterol‡ (mmol/liter)	5.5	(1.1)	5.6	(1.1)
History of hypertension (%)	26		18**	
Current smokers (%)	58		33**	
No. of cigarettes/day (among smokers)	25	(15)	18**	(12)
Angina pectoris (%)	14		5**	
Diabetes mellitus (%)	8		4**	
Family history of CHD† (%)	58		46**	
Alcohol use (%)	80		83	
Body mass index (kg/m²)	26.5	(3.9)	25.9*	(3.4)

* $p < 0.01$, **$p < 0.001$.
† SD, standard deviation; CHD, coronary heart disease.
‡ 520 cases and 586 controls.

To evaluate heterogeneity of results across centers, age- and smoking-adjusted odds ratios were calculated for each center separately for the center-specific 75th percentile versus 25th percentile of the selenium distribution among controls (table 3). For Germany, where the lowest selenium levels were observed, a significant inverse association was observed (odds ratio 0.62, 95 percent confidence interval 0.42–0.91). The odds ratios for the other centers ranged between 0.77 and 1.35 and were not significantly different from 1.0. The interaction of selenium with center was not significant ($p = 0.31$).

Next, the age- and center-adjusted odds ratios of myocardial infarction were calculated for quintiles of the selenium distribution among controls (table 4). The odds ratio in the highest selenium quintile was 0.32 (95 percent confidence interval 0.20–0.53, p for trend < 0.0001). However, after additional adjustment for smoking, the odds ratio in the highest quintile was 0.63 (95 percent confidence interval 0.37–1.07, p for trend = 0.08). Inclusion of family history of coronary heart disease in this model only marginally affected the odds ratios for selenium. Addition of vitamin E to the model slightly decreased the risk estimates, and the trend over quintiles became borderline significant (table 4); additional inclusion of beta-carotene did not change the odds ratios. Although interaction terms of selenium (in quintiles) with either vitamin E or beta-carotene (dichotomized) did not improve the fit of the regression model ($p = 0.92$ and $p = 0.33$, respectively), the observed inverse association appeared to be more pronounced at low vitamin E levels. In persons with a vitamin E status below the median (< 203 μg/g), the odds ratio for myocardial infarction at the highest selenium quintile was 0.45 (95 percent confidence interval 0.23–0.90); in subjects with vitamin E status above the median, this odds ratio was 0.72 (0.37–1.39).

Selenium was also modeled as a continuous variable in the logistic regression models. The odds ratios for the 75th percentile level compared with the 25th percentile level were not significantly different from 1.0 after adjustment for smoking and antioxidants (table 4). When including only those centers that used pop-

TABLE 3. Median toenail selenium concentrations (in μg/g) by center and disease status and adjusted* odds ratios for myocardial infarction at high (75th percentile) vs. low (25th percentile) selenium level in the EURAMIC Study, 1991–1992

Center	No. of cases	No. of controls	Cases		Controls		75th vs. 25th percentile	
			Median	25–75%	Median	25–75%	OR*,†	95% CI†
Germany (Berlin)	75	96	0.430	0.378–0.467	0.452	0.420–0.511	0.62	0.42–0.91
Netherlands (Zeist)	62	56	0.458	0.417–0.497	0.491	0.433–0.541	0.93	0.59–1.46
Spain (Granada)	54	52	0.487	0.443–0.528	0.499	0.454–0.587	0.74	0.37–1.48‡
Spain (Malaga)	94	100	0.504	0.465–0.555	0.531	0.461–0.604	0.85	0.52–1.38
Scotland (Edinburgh)	41	32	0.498	0.453–0.580	0.574	0.510–0.636	0.77	0.38–1.55
Switzerland (Zürich)	57	74	0.546	0.501–0.638	0.605	0.531–0.685	0.78	0.49–1.25
Russia (Moscow)	91	98	0.633	0.547–0.724	0.643	0.553–0.715	1.14	0.80–1.64
Norway (Sarpsborg)	96	100	0.640	0.575–0.707	0.654	0.581–0.719	1.35	0.96–1.89
Finland (Helsinki)	57	62	0.783	0.699–0.903	0.834	0.769–0.919	0.87	0.54–1.41
Israel (Jerusalem)	56	59	0.872	0.745–0.950	0.868	0.781–0.957	0.99	0.61–1.61
Overall	683	729	0.553	0.465–0.692	0.590	0.489–0.716	0.92	0.74–1.13

* Adjusted for age and smoking by logistic regression.
† OR, odds ratio; CI, confidence interval.
‡ To reach convergence, never smokers and exsmokers were combined in the model for Granada.

ulation controls exclusively, the odds ratio was significantly decreased to 0.65 (0.45–0.94).

Odds ratios for the 75th to 25th percentile of selenium were also calculated separately for current smokers, former smokers, and subjects who had never smoked. The interaction term of selenium (μg/g) and smoking (three categories) in the logistic regression model was not statistically significant ($p = 0.18$). An inverse association was observed in current smokers, with an odds ratio of 0.86 (0.64–1.16), and former smokers (odds ratio 0.63 (0.43–0.94)), but not in never smokers (odds ratio 1.55 (0.87–2.76)). Additional adjustment for number of cigarettes smoked daily in current smokers changed the odds ratio to 0.97 (0.71–1.32).

DISCUSSION

In this multicenter case-control study, we observed no significant association between toenail selenium levels and risk of nonfatal acute myocardial infarction when smoking habits were taken into account. The overall data indicated an inverse trend; however, this appeared to be restricted to exsmokers in stratified analysis, which may be due to inadequate adjustment for former smoking habits. With respect to individual centers, only in Germany, where selenium levels were the lowest, were low selenium levels associated with a statistically significant increased risk of myocardial infarction. The overall inverse trend was more pronounced at low vitamin E status than at high vitamin E status; however, there was no significant interaction between selenium and either vitamin E or beta-carotene.

Toenail selenium represents a measure of dietary intake over a period of several weeks, close to a year before the nail was clipped (22). This measure will not be affected by the acute event of infarction. Major dietary sources are meat and grain products. Apart from the diet, smoking habits, gender, and possibly age are also predictors of toenail selenium levels (21, 32). We adjusted for smoking habits and age in the calculation of risk estimates.

Low response rates in some of the centers using population controls may have led to underrepresentation of smokers in the controls. The stronger association for centers using population controls exclusively compared with the overall population is an indication for such a phenomenon. However, we are not convinced that there is such selection bias because the type of control selection did not affect the estimates for the association between beta-carotene and acute myocardial infarction, which we have reported previously (24), although beta-carotene is also an antioxidant that is affected by smoking status. It is also not very likely that selection bias would result in the controls having greater dietary intake of selenium, given the sources of selenium. For beta-carotene, it would be more likely that selection bias would lead to greater intakes, through vegetables, in the population controls. As stated previously, there was no indication of such selection bias in our beta-carotene results.

Norway and Russia were the only countries with a risk estimate greater than 1. For other centers, the odds ratios varied between 0.62 and 0.99, unrelated to sources of controls. Some selection bias cannot be excluded, but the direction is likely to be different for different centers. Therefore we feel it is prudent to base our conclusions on the pooled data, not on selected subsets.

Evidence for an association between serum selenium and risk of coronary heart disease has not been conclusive so far. An early report of an inverse asso-

TABLE 4. Odds ratios and 95% confidence intervals of risk of acute myocardial infarction by quintiles and for the 75th–25th percentile contrast of toenail selenium concentration in the EURAMIC Study, 1991–1992

Quintile	Median level of selenium (μg/g)	Age and center adjusted (*n* = 1,401)					Age, center, and smoking adjusted (*n* = 1,401)					Age, center, smoking, and vitamin E adjusted (*n* = 1,309)					Age, center, smoking, vitamin E, and β-carotene adjusted (*n* = 1,308)				
		OR*	95% CI*	Test for trend	75th–25th percentile		OR	95% CI	Test for trend	75th–25th percentile		OR	95% CI	Test for trend	75th–25th percentile		OR	95% CI	Test for trend	75th–25th percentile	
					OR	95% CI				OR	95% CI				OR	95% CI				OR	95% CI
1	0.432	1.0					1.0					1.0					1.0				
2	0.509	0.77	0.55–1.07				0.94	0.65–1.34				0.92	0.63–1.34				0.90	0.61–1.31			
3	0.590	0.48	0.33–0.70				0.64	0.43–0.96				0.68	0.45–1.03				0.69	0.46–1.05			
4	0.686	0.51	0.34–0.76				0.78	0.51–1.21				0.76	0.48–1.19				0.77	0.49–1.22			
5	0.862	0.32	0.20–0.53	<0.0001	0.88	0.55–0.84	0.63	0.37–1.07	0.08	0.92	0.74–1.13	0.59	0.34–1.02	0.05	0.91	0.73–1.14	0.60	0.34–1.05	0.07	0.92	0.74–1.16

* OR, odds ratio; CI, confidence interval.

ciation between serum selenium and coronary heart disease (4) has been confirmed by two prospective studies (3, 5). Virtamo et al. (3) reported an increased risk of cardiovascular death (relative risk 1.6) but not of myocardial infarction (relative risk 1.1) at serum selenium levels less than 45 μg/liter, which is extremely low. The more recent study by Suadicani et al. (5) shows an adjusted relative risk of ischemic heart disease of 1.55 at levels less than 1 μmol/liter (about 79 μg/liter). Adjustment for smoking was done for the categories never, ex-, and current smokers, which may leave some residual confounding. Several other prospective studies have found no association between serum selenium and coronary heart disease (7–10). In two of these nested case-control studies (9, 10) reporting relative risks of 1.0 for serum selenium less than 1.45 μmol/liter and 0.9 for levels less than 45 μg/liter, respectively, subjects were matched on smoking habits. Kok et al. (8) reported a relative risk of 1.1 for coronary deaths at low selenium levels. Toenail selenium levels of myocardial infarction cases were found to be significantly lower than in controls in a study in the Netherlands (33); the adjusted odds ratio for myocardial infarction at the lowest quartile of toenail selenium compared with the highest was 4.5 (95 percent confidence interval 1.3–15.7). Smoking was modeled as a dichotomous variable, so in this study as well, residual confounding by smoking may be possible. A case-control study from New Zealand (6) reported an increased risk of myocardial infarction at low selenium levels, which was confined to smokers. Again, this finding might be due in part to insufficient adjustment for smoking behavior (smoking yes/no). Moreover, serum selenium levels were determined in blood drawn directly after the infarction, which may have affected the levels in cases, although the authors report results from a pilot study showing no decrease of serum selenium immediately after the event.

The range of toenail selenium levels observed in this European study is much larger and levels are lower than those reported for different areas in the United States. The median levels of our lowest and highest quintile vary by a factor of 2. Hunter et al. (21) reported the lowest levels (adjusted for age, smoking, and supplement use) among participants in the Nurses' Health Study in Maryland (0.770 μg/g) and the highest in Texas (0.877 μg/g). Overall average levels for the United States were 0.801 μg/g for non-supplement users and 0.906 μg/g for supplement users, which were comparable with the highest quintile of our European population.

In conclusion, it appears that the increased risk of acute myocardial infarction at low levels of selenium intake is largely explained by cigarette smoking; vari-

ability in selenium status does not appear to be an important determinant of risk of myocardial infarction at the levels observed in a large part of Europe and in the United States.

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